Radioimmunolocalization of Human Gastric Carcinoma Xenografts SY 86 B and SY 86 D with $^{131}$I-Labeled Monoclonal Antibody

Xue Song CAO, Guang Yu YANG, Yin Chang ZHANG and Hiroshi KAWASAKI*

Cancer Institute, China Medical University
5-3, Nanjing Street, Heping District,
Shenyang, Liaoning, People’s Republic of China
*The First Pharmacy University
Tamagawa-cho, Minami-ku, Fukuoka-shi 815, Japan

Received July 4, 1990

The suitability of individual MAb for application in vivo should be carefully confirmed.

The monoclonal antibody GL-013, with specific binding reactivity in vitro to human tumors of the gastrointestinal tract, was radioiodinated and injected intraperitoneally into nude mice bearing human gastric carcinoma xenografts SY 86 B (moderately differentiated glandular adenocarcinoma) and SY 86 D (signet ring cell carcinoma). Whole body scintigraphy indicated tumor localization with $^{131}$I-labeled MAb GL-013, but not with $^{131}$I-labeled normal mice immunoglobulin. The best tumor contrast was obtained between days 3 and 7 after injection.

As confirmation of the imaging results, $^{131}$I-GL-013 preferentially localized in tumor tissue compared with normal tissue and $^{131}$I-GL-013 gave a higher tumor uptake ratio than the control $^{131}$I-NMIgG (at day 9 after injection), as determined by tissue counting of radioactivity. These results demonstrate that MAb GL-013 localizes in xenografts SY 86 B and SY 86 D and the possible clinical application of MAb GL-013 to radioimmunolocalization.

Key Words: monoclonal antibody, gastric cancer, nude mouse, radioimmunolocalization

An immunohistological study along with other tests using culture cells as targets do not require the use of the monoclonal antibody (MAb) for tumor targeting in vivo. The suitability of each MAb for application in radioimmunolocalization of tumors must be carefully confirmed.

In this paper, we investigated the localizing ability of $^{131}$I-labeled MAb GL-013 in human gastric carcinoma xenografts of two histological types.

1. Materials and Methods

1.1 Preparation and radiolabeling of MAb GL-013

The details for the generation and screening of hybridoma cell line. GL-013 have been reported

Briefly, the cells of metastatic lymph nodes from a patient with gastric cancer were used to hyperimmunize BALB/C mice. MAb GL-013 was purified from ascitic fluid by ammonium sulfate precipitation followed by DEAE chromatography.

MAb GL-013 and normal mouse IgG (NMIgG) with $^{131}$I were obtained by the Chloramine-T method. Briefly, the MAb GL-013 (75 µg) was mixed with 37 MBq of radiolabel and 10 µl Chloramine-T (2 mg/ml) in 0.3 M PBS, pH 7.4, for 2 min, after which saturated tyrosine was added to stop the reaction. Specific activities of radiolabeled MAb GL-013 and NMIgG ranging from $1.85 \times 10^5 - 4.44 \times 10^5$ Bq per µg of protein were determined.

1.2 Mice and xenografts

Human gastric cancer xenografts in nude
mice designated as SY 86 B and SY 86 D were established and passaged in our laboratory. The histological type of tumor SY 86 B is moderately differentiated tubular adenocarcinoma, while the histological type of tumor SY 86 D is signet ring cell carcinoma. Using a tracer, freshly excised tissue of xenograft SY 86 D was transplanted S.C. into the dorsal region of adult nude mice. The mice were used when the tumor reached a size of 1–2 cm in diameter. All the received 0.1%(V/V) KI in their drinking water throughout the experiment beginning 48 h before radiolabeled antibody administration.

1.3 Antibody binding assay
Labeled MAb GL-013 and control NMIgG were examined for binding activity in vitro using SY 86 B tumor cells and healthy human lymphocytes as targets. Constant numbers of SY 86 B tumor cells (1×10^5) and human lymphocytes (6.75×10^5) were incubated with various quantities of ^125^I-labeled MAb GL-013 in 100 µl of PBS buffer in wells of microtiter plates for 2 h at 37°C. The cells were then washed 3 times in buffer and the radioactivity in the pellet was determined with a gamma counter.

1.4 External scintigraphy
For imaging studies, 3.7–7.4 MBq of ^131^I-labeled MAb GL-013 or ^131^I-labeled NMIgG were injected i.p. into each mouse. Mice were sedated and placed in the prone position. Scintigraphy was performed daily using a rectilinear scintillation camera FTS-122 for 7 days after injection. On the ninth day post i.p. inoculation, the mice were bled and dissected. Tumors, blood and visceral organs were weighed and assayed for radioactivity. The tumor/non-tumor ratio was calculated.

### Table 1 In vitro binding reactivity of radiolabeled MAb GL-013

<table>
<thead>
<tr>
<th></th>
<th>^125^I-GL-013 MAb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1×</td>
</tr>
<tr>
<td>SY 86 B Ca cells</td>
<td>19.346±2.568</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3.971±149</td>
</tr>
<tr>
<td>Ratio</td>
<td>4.87</td>
</tr>
</tbody>
</table>

2. Results

2.1 In vitro binding reactivity of radiolabeled MAb GL-013
Table 1 shows iodinated MAb GL-013 to have higher binding reactivity to cells of xenograft SY 86 B than to human lymphocytes.

2.2 External scintigraphy
The images in Figs. 1 and 2 are representative of all four animals injected with ^131^I-labeled MAb GL-013 and show increased radioactivities in areas where xenografts SY 86 D are located. The best tumor contrast was obtained between 3 days and 7 days after injection. At 3 days after injection of ^131^I-labeled MAb GL-013 into a nude mouse bearing SY 86 B, scans, obtained before and after the visceral organs were taken out, showed the same well defined areas of increased radioactivities. None of the four xenografts were visible after injection of ^131^I-labeled NMIgG.

2.3 In vivo distribution of ^131^I-labeled MAb GL-013
Figures 3 and 4 show the tumor/non-tumor ratio for ^131^I-labeled MAb GL-013 and ^131^I-labeled NMIgG injected into nude mice bearing xenograft SY 86 B or SY 86 D. MAb GL-013 was preferentially localized both in xenograft SY 86 B and xenograft SY 86 D as evident from the much higher tumor/non-tumor ratios for ^131^I-labeled MAb GL-013 than for ^131^I-labeled NMIgG.

3. Discussion
Detection of the sensitivity of radioimmunolocalization has not been improved much by the monoclonal antibody. This may partially be due to the extreme specificity of MAbs. One study has reported a higher tumor
Fig. 1 Total body photoscans of a nude mouse bearing SY 86 D human gastric carcinomas in the dorsal region, taken 7 days following injection of 131I-labeled MAb GL-013.

Fig. 2 Total body photoscan of a nude mouse bearing SY 86 B human gastric carcinomas in the dorsal region, taken 3 days after injection of 131I-labeled MAb GL-013.

Fig. 3 Tumor/non-tumor ratio obtained from nude mice bearing tumor SY 86 D following the injection of 131I-labeled MAb GL-013 or 131I-labeled NMigG. ( ), NMigG, mean value of 3 tumors; ( ), MAb GL-013, mean value of 2 tumors. p<0.05(except thyroid gland)

Fig. 4 Tumor/non-tumor ratios obtained from nude mice bearing tumor SY 86 B following the injection of 131I-labeled MAb GL-013 or 131I-labeled NMigG.
to non-tumor ratio for MAb 3G9 injected into nude mice xenografted with the cell line M85, compared with those xenografted with the tissue of gastric carcinoma.

However, in the light of heterogeneity in the expression of antigenic determinants, carcinomatous tissue xenografted on nude mice is similar to carcinomas in patients. Thus, nude mice xenografted with carcinomatous tissue should be better suited for selecting MAbs capables of tumor targeting in vivo.

In this paper, in nude mice xenografted with human gastric carcinomatous tissue, preferential localization was shown for both signet ring cell carcinoma and moderately differentiated tubular adenocarcinoma with 131I-labeled MAb GL-013. Xenograft SY 86 B had a high spontaneous metastatic rate in nude mice and thus preliminary work indicates MAb GL-013 to possibly be capable of localizing more metastatic tumor sites and to be useful for improving the sensitivity of radioimmunolocalization of human gastric cancer following application in clinical diagnostic trials.

References

要 旨

ヒト胃癌の移植癌における放射性免疫学的局在性について

Xue Song CAO, Guang Yu YANG, Yin Chang ZHANG, 川崎 宏*

中国医科大学腫瘍研究所
5-3, Nanjing Street, Heping District,
Shenyang, Liaoning, P.R. China
* 第一薬科大学病態生理学教室 815 福岡市南区玉川町 22-1

単クローナ抗体GL-013と131Iをラベルしてヌードマウスのヒトの胃癌SY 86 B (differentiated carcinoma)とSY 86 D (signetring cell carcinoma)移植癌における放射性免疫学的局在性の定量を行った。

( 24 )